



Role of Notch Signaling in Colorectal Cancer

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Abstract

Notch signaling is a simple pathway in its mechanism and complex because it activates many genes at transcriptional level. Notch plays an imperative role in embryogenesis and progenitor/stem cell maintenance and critically preserves the balance between a cell proliferation, differentiation, and apoptosis. Mutation and deregulation of Notch will be a reason for many diseases including cancer. Aberrantly expressed Notch involves in the carcinogenesis of many cancers such as colorectal cancer (CRC), breast cancer, pancreatic cancer, prostate cancer, liver cancer, cervical cancer, Ewing sarcoma, Kaposi's sarcoma, lung cancer, ovarian cancer, and lymphoma. Among these, Notch plays a major role in CRC from development to metastasis. CRC occurs when the lining of colon epithelial cells becomes neoplasm, and this happens when the poise is missing between the normal and cancerous condition due to the overexpression of Notch. In CRC Notch promotes the stemness and epithelial to mesenchymal transition (EMT) which are requiring in aberrant crypt formation, invasion, and metastasis, respectively. Moreover, overexpression of Notch in CRC is connected with poor prognosis and chemoresistance. Notch counteracts with Hippo/YAP, WNT, NFkB, PI3K/Akt, and EGFR pathways for tumor initiation and development. Tumors are dependent on angiogenic switch for development and invasion which is activated by Notch ligand Dll-4 by overexpressing vascular endothelial growth factor (VEGF). This review will be focusing on the uniqueness of Notch in CRC.

Keywords

Notch · CRC · Chemoresistance · Transcription factor and EMT pathway

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21.1 Introduction

Colorectal cancer (CRC) is the third most common cancer and reason for 610,000 deaths per year worldwide. The death in CRC is mostly due to liver metastasis, tumor recurrence, chemoresistance, and cancer stem cells (CSCs). Besides, the deregulated signaling pathways include WNT, Notch, EGFR, and TGF- β acting a key role in CRC development [26]. Among these, Notch pathways poise a pivotal role in CRC tumor initiation, tumor recurrence, metastasis, poor prognosis, chemoresistance, and maintains of CSCs [8]. Notch is a single transmembrane receptors composed of four receptors Notch 1–4, along with five canonical ligands, Jagged (Jag)1, Jag2, Delta like (DII) 1, DII3, and DII4. Notch plays an imperative role in embryogenesis and progenitor/stem cell maintenance and critically preserves the balance between a cell proliferation, differentiation, and apoptosis [8]. Mutation or deregulation of Notch signaling pathway leads to many diseases including cancer. Aberrantly expressed Notch involves in the carcinogenesis of many cancers such as CRC, breast cancer, pancreatic cancer, prostate cancer, liver cancer, cervical cancer, Ewing sarcoma, Kaposi's sarcoma, lung cancer, ovarian cancer, and lymphoma [23]. Among these, Notch role in CRC is crucial from its initiation to metastasis. So understanding the role of Notch in CRC will pave a way to find new therapeutic targets for the treatment of CRC.

21.2 Notch Structure

Notch is a single transmembrane juxtacrine-mediated signaling pathway. Notch family consists of four type-I transmembrane receptors, Notch 1–4. These are synthesized in precursor form and undergo subsequent cleavages to form mature active Notch receptor. The first cleavage is by furin-like convertase at trans-Golgi apparatus and forms extracellular domain, intracellular domain, and transmembrane domain. The extracellular domain is made up of 36 EGF-like repeats. The C-terminal of the EGF repeats consists of three cysteine-rich lineage defective-12 (LIN-12) and Notch repeats (LNR) which prevents receptor activation in the absence of ligand. Following this, it consists of heterodimerization domain and transactivation domain. Intracellular domain consists of RBPJ-associated molecule (RAM) domain, two nuclear localization signals, 6 ankyrin (ANK) repeats, and a PEST (proline-glutamine-serine-threonine) sequences which are collectively called as Notch intracellular domain (NICD) (Fig. 21.1) [3, 11, 15]. The sequence of proteolytic cleavage, cleavage site, and enzymes involved in cleavage are given in Table 21.1.

21.3 Notch Mechanism

Notch is a single transmembrane receptor with extracellular, transmembrane, and intracellular regions, and it is activated by sequence of proteolytic cleavages. The pathway activation is initiated by binding of one of the five (Jag1 and Jag2, DLL-1,

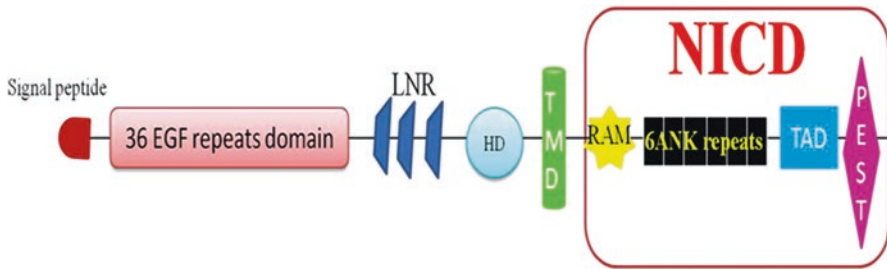


Fig. 21.1 Notch structure: EGF, epidermal growth factor; LNR, Lin12 Notch repeats; HD, heterodimerization domain; TMD, transmembrane domain; ANK, ankyrin repeats; TAD, transactivation domain; and PEST, proline-glutamic acid-serine-threonine

Table 21.1 Notch cleavage site and enzymes

Cleavage	Cleavage site	Enzyme	References
S1 cleavage	1664/1665	Furin-like convertase	[3]
S2 cleavage	1710/ 1711	A disintegrin and metalloprotease (ADAM)	[3]
S3 cleavage	1743/1744	γ -secretase	[3]

DLL-3, and DLL-4) ligands which leads to the conformational changes in Notch receptors. Subsequently, S2 cleavage site is exposed for ADAM to remove the extracellular region. Following this, S3 cleavage occurs by γ -secretase (complex of nicastrin and presenilin-1) which removes the transmembrane region and releases Notch intracellular domain (NICD). The NICD is accumulated in cytoplasm and then translocated to the nucleus by concentration gradient where it binds with transcriptional regulator termed as C-protein binding factor 1 [(CBF1) also known as RBP-Jk or CSL for CBF1/Su(H)/Lag1]] leading to the displacement of corepressor and brings coactivators such as MAML-1 to activate the expression of hairy and enhance of split (Hes) and Hes-related repressor protein (Hey) families [1, 6, 7] (Fig. 21.2).

21.4 Notch and Transcription Factors

Once ligand binds to the Notch receptor, it can activate proteolytic cleavage mediated by gamma-secretase lead to release NICD. NICD is acting as a transcription coactivator, but it can't bind to DNA, because it doesn't have a zinc finger domain. Consequently, they can recruit RBP-J and MAML1–3 to activate transcription genes through binding with DNA. Once they are formed, it can recruit chromatin remodeling complex, which consists of histone deacetylase or histone acetylase proteins. In the absence of ligand or transcription coactivator of NICD, the RBP-J acts as a repressor. Notch activates many transcription factors directly and indirectly such as HES1, HEY1, TGF β , NF- κ B, snail, slug, PTEN, and cyclin D3 [14].

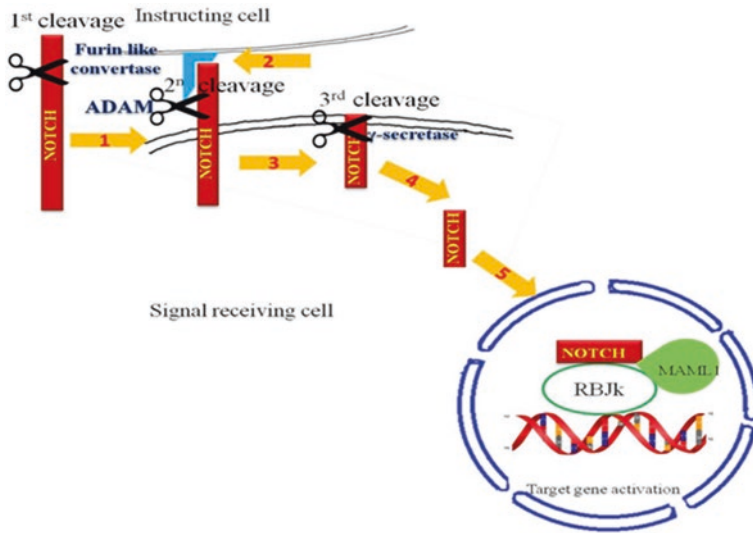


Fig. 21.2 Notch signaling pathway. 1. Notch receptor is cleaved by furin-like convertase in trans-Golgi to produce mature Notch receptor. 2. One of the five (Jag1, Jag2, Dll-1, Dll-3, and Dll-4) binds from instructing cell to activate signaling pathway. 3. Exposure of S2 cleavage site and cleaved by ADAM enzyme to extracellular region. 4. S3 cleavage carried out by γ -secretase to release Notch intracellular domain (NICD). 5. NICD is then translocated to nucleus and activates target gene expression

21.5 Notch in Colorectal Cancer

Colon is the part of digestive system is lining with absorptive and secretory epithelial cells interspersed with deep crypts which consists of 2000 cells with 5–10 stem cells [19]. CRC is identified with aberrant crypt formation intervened by Notch pathway. Notch signaling regulates the cell proliferation and differentiation of epithelial cells to absorptive or secretory cells, maintaining apoptosis in the colon. The deregulated Notch will fail to spot the poise and leads to the formation of cancer [26]. Notch-mediated tumor initiation mechanism in CRC is not well understood, but the possible reason will be overexpression of mutated Notch receptors, ligands, and Notch signaling interplay between other signaling pathways. Notch is highly expressed in the primary stage of CRC relatively than the later stage. Notch1 and Hes1 are highly expressed in primary and metastatic CRC than colonic mucosa [19]. The other reasons could be (i) mutation of E3 ubiquitin ligase which is responsible for NICD degradation; (ii) mutation in APC leads to the overexpression of NICD [28]; (iii) the decreased expression of atonal homolog1 (Atoh1) which is essential for intestinal secretory cell commitment; and (iv) Hes1, the direct target of Notch, is repressor for KLF4 which inhibits CRC cell proliferation when overexpressed [2]. Other than this Notch interplays with many signaling pathways in CRC such as WNT, NF- κ B, EGFR, and TGF β . Notch signaling can be an oncogenic or tumor suppressor; it depends on the context of cells in where cancer occurs [24].

21.5.1 Notch Interplay with Signaling Pathways in CRC

Wnt/ β -catenin signaling is the essential pathway which is highly articulated in 80% of sporadic CRC, and it is the main reason for inherited familial adenomatous polyposis (FAP)-related CRC [23]. Wnt/ β -catenin signaling is the key pathway involved in the transformation of epithelial cells into self-renewing CSCs for CRC development. Wnt/ β -catenin signaling activates its target Jag1 that trigger the Notch and increase its expression in CRC. Most of the CRC carries the mutation of APC which is the component of Wnt signaling pathway. In Apc-mutated condition, Notch signaling is requisite for consequent development of CRC [4, 26, 27]. PI3/AKT pathway is upregulated in 40% of human CRC, and it is important for cell survival and growth. Notch activates its target HES1 which inhibits PTEN leading to the activation and overexpression of PI3/AKT pathway in CRC. Notch involves in colonic carcinogenesis by stimulating EGFR pathway. Notch signaling arbitrated activation of transcription factor NF- κ B in CRC results in chemoresistance of CRC cells [10]. Notch also inhibits the activity of transcription factor TGF- β pathway which is important for tumor suppressor and cell growth inhibition. In CRC, TGF- β -mediated induction of Jag1 results in the overexpression of Notch [30]. Angiogenesis is the formation of new blood vessels which is an important characteristic of growing tumor. Inhibiting or disrupting angiogenesis will be the great therapeutic way to inhibit or treat cancer [13]. Jag1 Notch ligand activates the Notch-dependent angiogenesis by activating VEGF in CRC [21]. Notch signaling upregulates the expression of anti-apoptotic genes including Bcl2 and BclXL. With these Notch also upregulates the expression of cyclin D1 and p21/WAF1 which are involved in cell cycle regulation and cell proliferation [23].

21.5.2 Notch Interaction with EMT Pathway

An epithelial mesenchymal transition (EMT) pathway plays an important role in key biological process of embryogenesis and metastatic cancer development [31]. The basement-degraded epithelial cells migrate to form new types of mesenchymal cells in order to increase the motility and invasion [16]. During this EMT process, the epithelial cell loses the cell junction followed by the reorganization of the actin cytoskeleton to initiate the transition by the relocation of epithelial markers such as E-cadherins and integrins from cell membrane to the nucleus. These could gain the expression mesenchymal markers such as N-cadherins, vimentin, and fibronectin [30]. Moreover, the growing report indicates that cancer stemness and chemoresistant mechanism are achieved by high rates of EMT signaling pathway [8], especially its interaction with oncogenic signaling pathways like MMP-3, BCL9-2, EGFR, met, gooseoid, kaiso, TGF- β , FOXC2, GSK-3 β , Smad-3, Pez, Snail1, Snail2, ILK, and Notch [30]. Interestingly, the recent report revealed that the growing incidence of colon cancer is due to overexpression of Notch and its regulation of EMT pathway [9]. Notch signaling can regulate Snail1 expression through the induction of hypoxia inducible factor 1 α (HIF-1 α). Subsequently the HIF-1 α binds the promoter region of LOX (lysyl oxidase) for stabilization of Snail1 and its

transcription. Likewise Notch interact Snail2 leads to repression of E-cadherin and β -catenin activation [25]. Moreover, targeting of Notch signal leads to downregulation of EMT and induction of p21 in human colon cancer cells [5].

21.5.3 Targeting Notch Signal for CRC Treatment

The gamma-secretase inhibitors (GSI) compounds treated CRC showed inhibition of the γ -secretase, which is a mandatory enzyme to activate Notch internal signaling, but it induces extracellular signal-regulated kinases (Erk) for continued cell proliferation. However, the GSI combined with cisplatin drug-treated cells exhibited the downregulation of Erk and activation of cell death [2]. The combined treatment of biphenolic compound and ionizing radiation (IR) treated in CRC results revealed the downregulation of the Notch, CRC stem cell protein marker DCLK1, and downstream target gene of Hes1. These downregulation genes can activate the apoptosis pathway. A similar result has observed in tumor xenograft animal model [22]. Interestingly the flavonoid compound epigallocatechin-3-gallate-treated cells has showed promising activity toward the inhibition of β -catenin, but quercetin showed weak inducer for downstream target genes reported in human colon carcinoma cell line HT29 [20]. The report of [12] indicates that the bioactive compound of Withaferin-A isolated from *Withania somnifera*-treated colon cancer cells exhibited downregulation of Notch signal by induction of c-Jun-NH2-kinase-dependent apoptosis. These mechanisms can reduce the expression of Akt/NF- κ B/Bcl-2 and mammalian target of rapamycin signaling components (pS6K and p4E-BP1). Likewise, a γ -secretase inhibitor can induce the differentiation of goblet cell from the proliferative crypt in mice carrying mutated gene for an Apc tumor suppressor. Similar result has seen in blocking of Notch signal with γ -secretase inhibitor [29]. These reports further support the interaction of Wnt and Notch signaling pathways. Moreover, the Jagged1 was silenced by lentiviral Jagged1-shRNA resulted in decrease of cell viability. Besides, the knockdown studies showed the induction induced cell cycle arrest at G0/G1 phase with reduced expression of cyclin D1, cyclin E, and c-Myc in vitro colon cancer cell. However, the knockdown studies of xenograft mouse model have reflected the same results of in vitro [6]. The small molecular inhibitor FLLL32-treated colon cancer stem cell has seen the inhibition of STAT3 through the downregulation of survivin, Bcl-XL, and Notch signal. Moreover, the significant inhibition of tumorsphere formation and induction of cleaved caspase-3-mediated apoptosis observed in small molecule-treated cells are compared to curcumin-treated cells [17].

Curcumin analogue GO-Y030 treatment has shown the inhibition of activated STAT3 followed by reduction of cyclin D1, survivin, Bcl2, Notch, and p-RP in colon cancer cells. These reductions can facilitate the cleaved caspase-3-mediated apoptosis. Moreover, GO-Y030 treatment reduces the tumor growth in mouse xenografts with SW480 and HCT-116 colon cancer stem cells in dose-dependent studies [17]. However, Meng et al. [18] reported that the oxaliplatin, 5-fluorouracil (5-FU), and SN-38 compound-treated cell revealed the induction of the Notch-1

intracellular domain (NICD)-mediated chemoresistance toward cancer cell progression through the activation of Y-secretase. Subsequently, silencing of Y-secretase subunit nicastrin by small interfering RNA (siRNA) prevented activation of NICD after the treatment of oxaliplatin. Concurrently, the silencing or blocking of NICD by sulfonamide GSI (GSI34)-treated colon cancer cells sensitizes to chemotherapy compounds through downregulation of phosphoinositide kinase-3/Akt. The inhibition of Notch signaling leads to suppression of tumor invasion and intravasation in genetic depletion Aes in ApcD716 mice. These reports further confirm the inhibition of Notch potential target for control and treatment of metastasis colon cancer [28].

21.6 Conclusion

Notch plays a major role in normal development and cancer condition. Its role in CRC cancer is very important from cancer progression to metastasis. This review explained about Notch and its transcription factor role in CRC mainly in metastasis and targeting Notch pathway in CRC. Targeting Notch will be a great therapeutic source to treat the CRC.

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